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PILOT CONTROL PROJECT OF NUCLEOPOLYHEDROSIS VIRUS AND *BACILLUS THURINGIENSIS* TO CONTROL DOUGLAS-FIR TUSSOCK MOTH POPULATIONS IN IDAHO - 1974[1/]

by

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ABSTRACT

A pilot control project designed to evaluate two microbial insecticides, the bacterium *Bacillus thuringiensis* and a nucleopolyhedrosis virus, against epidemic populations of Douglas-fir tussock moth was planned for two test sites in northern Idaho in 1974. Tests were aborted due to a natural decline of tussock moth population and/or their not reaching expected population levels. Problems encountered in formulation of the microbial insecticides, insect population data, and potential for damage in 1974 are discussed.

INTRODUCTION

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., periodically reaches epidemic levels in the Northwest. Detailed histories of outbreaks in Montana, Idaho, Oregon, and California are discussed by Wickman et al. (1973) and Tunnock (1973). Past outbreaks have usually persisted for 2 to 4 years at which time they decreased to endemic levels primarily from epizootics of a nucleopolyhedrosis virus.

Field tests of two microbial agents, a nucleopolyhedrosis virus and the bacterium *Bacillus thuringiensis* Berliner, applied at various treatments

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by helicopter to 20-acre plots in eastern Oregon in 1973, showed considerable promise as control agents against the Douglas-fir tussock moth. These tests were the basis of a pilot control project designed to evaluate operational feasibility of using these materials as an alternative to DDT for control of tussock moth infestations.

The current Douglas-fir tussock moth outbreak in Idaho was believed to contain criteria needed for a definitive pilot project with virus and bacteria. These criteria as described by Stelzer and Neisses (1973) were:

1. Relatively high Douglas-fir tussock moth larval densities.

2. A population in the release phase of the outbreak cycle (as defined by Wickman et al., 1973).

3. A population with a low level of natural virus associated with the overwintering eggs.

OBJECTIVES

The objectives of this pilot test were to evaluate the effectiveness of an aerial application of Dipel (*Bacillus thuringiensis*) and the nucleopolyhedrosis virus in reducing Douglas-fir tussock moth larval populations and protection of foliage when applied under operational conditions.

Secondary objectives of the study were to:

1. Investigate the correlation of spray deposit with larval mortalities.

2. Determine incidence of parasitism of the Douglas-fir tussock moth in treated and untreated areas under the limitations of the sampling schedule.

METHODS

Locations of Study Areas

This pilot control project was established at two locations--Coeur d'Alene Mountain south of Coeur d'Alene, Idaho, on the Idaho Panhandle National Forest, and Lookout Butte south of Lowell, Idaho, on the Nezperce National Forest. Both materials were to be applied at each location.

Criteria for plot selection were that plots would have good accessibility and somewhat natural boundaries, relatively high Douglas-fir tussock moth populations, and defoliation more or less uniform among the plots. The sampling portion of the plots would be located a sufficient distance from the next plot to minimize contamination by spray drift. Distances would vary with terrain and wind patterns.

Treatments to be applied to epidemic Douglas-fir tussock moth populations included the most promising of each of the *Bacillus thuringiensis* and nucleopolyhedrosis virus formulations tested in eastern Oregon in 1973. On a per acre basis, dosages were as follows:

Bacillus thuringiensis

Dipel wettable powder[3/]	1.0 pound
Cargills Insecticide Base (molasses)	.5 gallon
Brilliant sulfur yellow dye	7.6 grams
Water to make 2 gallons spray	

Virus

100 billion polyhedra nucleopolyhedrosis virus	
Cargills Insecticide Base (molasses)	.5 gallon
NaOH buffer	26.4 grams
Brilliant sulfur yellow dye	7.6 grams
Sun screen	1.0 pound
Water to make 2 gallons spray	

Sample Design

The two areas were each divided into nine blocks (three virus, three *Bacillus thuringiensis*, and three controls). Blocks varied in size from 1,000 to 3,000 acres due to differences in natural boundaries. Treatments were assigned to blocks randomly. A total of approximately 20,000 acres were scheduled for treatment with Bt *(Bacillus thuringiensis)* and 7,000 acres were scheduled for treatment with virus.

Based on estimated sample variances and what was feasible manpower-wise, the selected sample design was to cluster sample taking two branches per tree, five trees per cluster, and 16 clusters per block for a total of 80 trees per block. Clusters were to be located throughout the spray area.

Thirty to 40 egg masses were tagged per spray block and examined every other day for hatch to determine when spraying would commence. Spraying would begin when 50 percent of the larvae had developed to second instar. After 50 percent of the egg masses had hatched, branch samples were collected from 20 trees in each spray block and larvae were removed from each branch and taken to the laboratory for instar determination. Instar development was measured daily when 20 percent of the larvae reached the second instar.

3/ Product of Abbott Laboratories, North Chicago, Illinois. (Mention of commercial names is for convenience only and does not imply endorsement by USDA.)

Pretreatment Evaluations

Egg masses were collected from 26 sections of land within the Coeur d'Alene Mountain treatment area and from 22 sections within the Lookout Butte area as part of the fall 1973 egg mass survey (Tunnock and Livingston, 1974). These were used for virus and egg parasite determination.

If an average of 5% or more of the larvae from an area was infected with naturally occurring virus, that section was deleted from the test site. Those sections with virus levels between 1 and 5% were evaluated further before deleting them from the test. Areas with 1% or less virus were to be treated. Alternative test sites were to be selected if virus levels were too high in the proposed test areas.

Method of Application

Application was to be by helicopter in all blocks scheduled for treatment with microbial insecticides.

RESULTS

Formulation of Microbial Insecticides

Examination of materials received to formulate the microbial sprays revealed that the molasses contained tiny particles of a solid material. These particles would not pass through 100-mesh screen which would have resulted in clogged or partially clogged nozzle screens on the aircraft spray system and an inconsistent flow rate.

Discussions with representatives of Cargills indicated that the solid material in the molasses was vermiculite. Santoquin, an additive to prevent fermentation formulated on a vermiculite base, had been added to the molasses. Contract specifications called for addition of liquid Santoquin. Attempts to screen a diluted molasses-water mix through a 100-mesh screen prior to spraying failed resulting in clogged nozzle screen within 20 minutes when tested in a simulated spray boom at 40 p.s.i. (figures 1 and 2). The material was rejected and plans were reluctantly made to substitute Biofilm, a commercial surfactant, for the molasses.

Three women filtering and screening Douglas-fir tussock moth body parts and hairs from the virus spray exhibited symptoms of contact dermatitis typical of exposure to mature tussock moth larvae.
Symptoms of dermatitis persisted for 2 weeks.

Decline of Tussock Moth Populations in Test Sites

Installation of spray blocks in the Selway test site in late May and early June indicated that tussock moth egg mass populations were somewhat lower than earlier egg mass surveys indicated. In addition, access

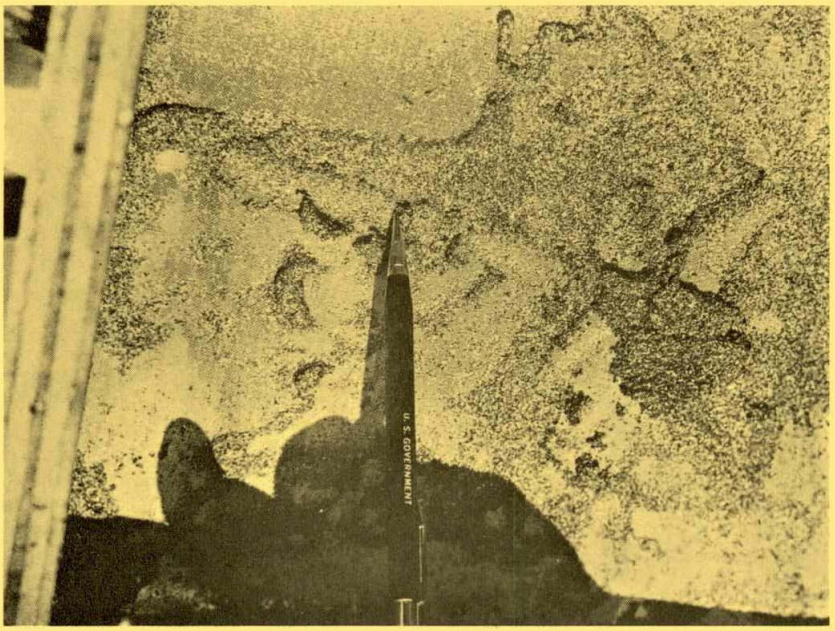

Figure 1.--Vermiculite debris from molasses-water mix on 100-mesh screen.

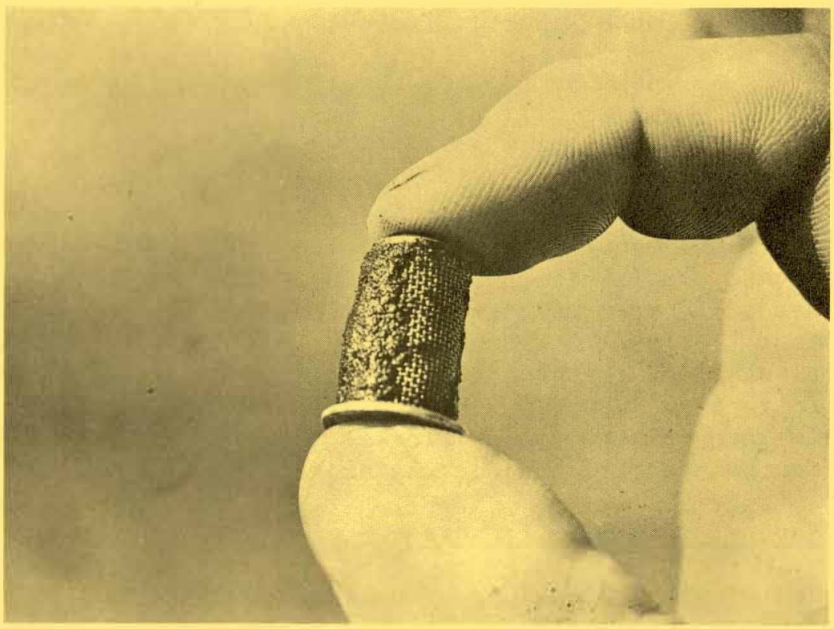

Figure 2.--Nozzle screen clogged with vermiculite after molasses mix passed through 100-mesh screen.

and acres of clearcut units within the test site made it virtually impossible to include nine blocks within the area available for testing. On June 4, a decision was made to eliminate the three virus test blocks and proceed with a design which included three Bt blocks and three check blocks.

A prespray larval sample taken June 24 indicated that tussock moth population densities were too low to support a valid pilot control project in the Selway test site; therefore, the entire Selway pilot project was aborted (Table 1). Larval development at this time was:

Instar	Percent
First	4
Second	83
Third	13

Table 1.--Prespray Douglas-fir tussock moth levels, Selway test site, Nezperce National Forest, Idaho, June 24, 1974

Block		Douglas-fir tussock moth larval density (insect/1,000 sq. in. foliage)
Lookout Butte	(spray)	3.14
Lodge Point	(spray)	.87
Tahoe Ridge	(spray)	7.35
Tahoe #2	(check)	1.18
Corral Hill #1	(check)	2.77
Corral Hill #2	(check)	5.61

Similar observations were being made on tussock moth populations in the Mount Coeur d'Alene test site. Prespray samples in late June showed Douglas-fir tussock moth populations were lower than the number needed to meet criteria established for Bt and virus for this pilot test (Table 2). Minimum population density levels established prior to start of the project were 20 larvae per 1,000 square inches of foliage in Bt spray blocks and 50 larvae per 1,000 square inches of foliage in virus spray blocks. Since these criteria could not be met, the entire project was aborted on June 29.

Natural Mortality Factors

Empirical observations by numerous individuals associated with the project indicated that the early decline of larval populations in the Mount Coeur d'Alene test site was due to a number of factors. These included low egg viability possibly due to climatic abnormalities which occurred in late winter or early spring; heavy populations of

Table 2.--Larval tussock moth population densities, Coeur d'Alene Mountain spray blocks, 1974

Plot name	Larvae per 1,000 square inches of foliage				Percent natural reduction (prespray to 35-day)
	Prespray	7-day	21-day	35-day	
	Dates sampled				
	6/27-29/74	7/3-12/74	7/22-29/74	8/5-13/74	
Virus I 301	--	0.8	0.7	0.1	87
Cottonwood Bt-801	7.0	3.2	.4	.1	99
Virus III 501	20.0	15.0	7.5	2.0	90
Turner Bt-401	45.0	12.2	2.9	.6	99
Beauty Bt-601	27.0	49.0	3.8	1.6	94
Pleasant Bt-701	16.0	3.5	3.2	1.5	91
Elk Mtn. Check 1-80	1.8	.9	.2	.2	89
Beauty Check 101	6.0	1.2	.8	.3	95
Fortier Check 201	10.0	8.0	1.2	.4	96
Average					93

an unidentified aphid which infested the new growth in grand fir and produced large volumes of honeydew which prevented first instar tussock moth larvae from feeding and entrapped young larvae when exposed to honeydew-covered foliage; and predation by ants. Honeydew-covered foliage resulted in dispersal of tussock moth larvae to new foliage or host trees. This resulted in mortality of some larvae as they did not reach suitable host trees.

After the project was aborted, tussock moth populations were monitored to determine the effect of natural factors on the remaining population in the Coeur d'Alene Mountain test site.

Two 15-inch branches were removed from each of 80 trees in each of the nine spray units at 10-day intervals following the prespray samples. When present, 10 larvae and/or pupae were collected from each tree each sample date and placed separately in Petri dishes, then returned to the laboratory. At the laboratory a piece of artificial media was placed in each Petri dish. The dish was labeled as to block, tree number, and date, then placed on a shelf for rearing. The following data were collected from these rearings: Number of larvae or pupae dying from unknown causes, number emerging as adults, and larval and pupal mortality resulting from hymenopterous and dipterous parasites and virus.

A total of 6,689 tussock moth larvae were reared to determine mortality from various causes. Of the tussock moths reared, 48.9% emerged as adults; 36.7% died from unknown causes; 9.2% died from hymenopterous parasites; 1.8% died from dipterous parasites; and 3.4% from virus (Table 3).

Table 3.--Douglas-fir tussock moth mortality in nine spray blocks near Coeur d'Alene, Idaho, during summer of 1974

Plot name	Total collected	Emerged as adults	Percent mortality from various causes			
			Unknown causes	Hymenop-terous parasites	Dipterous parasites	Virus
Virus I	70	45.7	21.4	25.7	1.4	5.7
Cottonwood	320	52.2	33.7	10.3	.3	3.4
Virus III	1,371	49.9	38.4	5.9	3.2	2.6
Turner	1,235	43.2	43.9	7.5	.6	4.8
Beauty Bt	1,695	54.3	31.6	10.8	.2	3.1
Pleasant	921	48.7	37.2	7.2	4.2	2.5
Elk Mountain	117	55.5	29.0	11.1	1.7	2.6
Beauty Check	239	48.9	33.5	15.5	0	2.1
Fortier	721	42.2	38.0	12.5	3.0	4.3
Grand total	6,689					
Averages		48.9	36.7	9.2	1.8	3.4

Parasitism by hymenopterans ranged from 0 to 26 percent (average 9.2%) with highest percent parasitism occurring in larvae collected in the 35-day sample. Larvae were mostly fifth and sixth instars by this time. Parasitism by dipterans ranged from 0 to 32 percent (average 1.8%) with highest parasitism occurring in the pupal sample. Mortality from virus ranged from 0 to 11 percent (average 3.4%) with highest larval viral mortality occurring in larvae collected during the 7-day sample.

Fall Egg Mass Survey

Each section of land infested with tussock moth, as determined by the 1973 fall egg mass survey except those included in the Bt field experiments conducted by the Pacific Northwest Experiment Station, was sampled for egg masses during September 1974.

A total of 18 sections were sampled. Sampling consisted of felling five Douglas-fir or grand fir and removing four whole branches from each of three crown levels (lower, mid, top). All new and old egg masses and cocoons were counted on each branch. Five egg masses, when present, were collected, placed in paper bags, and returned to the laboratory for evaluation of natural virus, egg viability, and egg parasitism.

Of the 18 sections sampled, four met pre-established treatment criteria of 0.1 egg mass per 1,000 square inches foliage (Table 4).

Table 4.--Douglas-fir tussock moth egg mass densities, Mount Couer d'Alene, Idaho, September 1974

Plot location			1973 egg masses			1974 egg masses			Average egg mass ratio density		
T.	R.	Sec.	Lower	Mid	Top	Lower	Mid	Top	1973	1974	Old:new
49N	2W.	2	0.127	0.096	0	0.483	0.576	0.706	0.096	0.552	1:5.750
49N	2W	4	.037	0	.104	.037	0	0	.042	.014	1:0.333
48N	2W	6	.057	.211	0	0	0	0	.110	0	1:0
49N	3W	7	0	0	.209	.030	0	0	.027	.013	1:0.481
49N	2W	8	.041	0	0	0	.035	0	.015	.015	1.1.000
49N	3W	14	0	0	0	0	.131	0	0	.057	1:0
49N	3W	15	0	0	.097	0	0	.194	.015	.029	1:1.930
49N	2W	17	0	.282	.792	.092	.169	.132	.233	.127	1:0.545
49N	2W	19	.076	0	0	0	.046	0	.033	.017	1:0.515
49N	2W	20	0	0	0	.083	.390	.225	0	.237	1:0
49N	3W	22	.063	.106	.261	0	.035	.131	.103	.030	1:0.291
49N	3W	23	.029	.115	.213	0	.115	.107	.085	.057	1:0.670
49N	3W	24	0	.281	.112	.070	.047	.112	.119	.068	1:0.571
49N	3W	25	1.260	.961	.135	0	0	0	1.160	0	1:0
49N	3W	26	0	.652	.117	.054	.130	0	.232	.073	1:0.314
49N	2W	29	0	.052	.149	0	.052	0	.041	.021	1:0.512
49N	2W	32	0	0	0	0	.178	.331	0	.115	1:0
49N	3W	36	0	.112	0	0	.056	0	.047	.024	1:0.511
Mean			0.094	0.159	0.188	0.047	0.109	0.107	0.131	0.085	1:0.649

A total of 34,138 acres qualified for treatment based on egg mass density counts in the Coeur d'Alene unit in 1973 (Tunnock and Livingston 1974). Based on the 1974 egg mass survey, less than 2,000 acres qualify for treatment. Only 1,200 acres of aerially visible defoliation actually occurred in 1974. It is anticipated that evaluation of virus, egg viability, and egg parasitism will further reduce this acreage.

Reconstitution of Polyhedrosis Virus

Virus formulated for aerial application will be recovered by lyophilization and stored for future testing. Estimated cost of lyophilization is $6,000.

DISCUSSION AND CONCLUSIONS

In light of the initial low population levels in some blocks and the high rates of larval mortality throughout the feeding period of Douglas-fir tussock moth larvae on Mount Coeur d'Alene, it is our opinion that the decision to abort this pilot project was sound. Little valid data supporting registration of these highly promising biological materials could have been obtained had the treatments been applied. It is important to note that similar population declines did not occur in other parts of the epidemic area in northern Idaho and most of the areas which were scheduled for treatment with DDT continued to meet the treatment criteria throughout the time the control project was being carried out.

Several problems were encountered in formulating the microbial insecticides, particularly with the vermiculite in the molasses. These problems can readily be overcome with close contract administration in future pilot control or operational projects involving microbial insecticides.

Population monitoring following the abort decision indicates that the nucleopolyhedrosis virus was not a major factor in the decline of this outbreak. Instead a complex of natural agents accounted for the decline and a low population is expected to persist in the area in 1975.

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